

# **EXHIBIT A197**

## Gene Expression Profiles of Serous, Endometrioid, and Clear Cell Subtypes of Ovarian and Endometrial Cancer

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**Abstract** **Purpose:** The presence of similar histologic subtypes of epithelial ovarian and endometrial cancers has long been noted, although the relevance of this finding to pathogenesis and clinical management is unclear. Despite similar clinical characteristics, histologic subtypes of cancers of the ovary and endometrium are treated according to organ of origin. This study compares the gene expression profiles of analogous histologic subtypes of cancers of the ovary and endometrium using the same genomic platform to determine the similarities and differences between these tumors.

**Experimental Design:** Gene expression profiles of 75 cancers (endometrioid, serous, and clear cell) of the ovary and endometrium, five renal clear cell cancers, and seven normal epithelial brushings were determined using a 11,000-element cDNA array. All images were analyzed using BRB ArrayTools. Validation was done using real-time PCR on select genes and immunohistochemical staining.

**Results:** Comparison across endometrial and ovarian cancers and serous and endometrioid tumors showed expression patterns reflecting their organ of origin. Clear cell tumors, however, showed remarkably similar expression patterns regardless of their origin, even when compared with renal clear cell samples. A set of 43 genes was common to comparisons of each of the three histologic subtypes of ovarian cancer with normal ovarian surface epithelium.

**Conclusions:** The comparison of the gene expression profiles of endometrioid and serous subtypes of ovarian and endometrial cancer are largely unique to the combination of a particular subtype in a specific organ. In contrast, clear cell cancers show a remarkable similarity in gene expression profiles across organs (including kidney) and could not be statistically distinguished.

Cancers of the uterus and ovary together account for 10% of new cancer cases and 7% of cancer deaths in women in the United States (1). Similarities in their histologic appearance have led to classification schemes that include serous, endometrioid, clear cell, and mucinous subtypes for both organs. However, the question of whether the presence of parallel subtypes in ovarian and endometrial cancer reflects common pathogenetic processes has not been clarified.

The development of endometrial cancer is thought to involve two pathways. The classic or type I pathway occurs in the setting of estrogen excess, is associated with atypical hyperplasia as a

precursor, and generally develops at an earlier age at a low stage and grade. In contrast, the alternative or type II pathway involves atrophic endometrium, proceeds through a precursor known as endometrial intraepithelial carcinoma, and presents in older patients and at a higher stage and grade (reviewed in ref. 2). The first pathway is associated with endometrioid histopathology, whereas the second is linked to the serous subtype. Molecular evaluations have supported the distinction between the two types of endometrial cancer, showing that type I tumors tend to have frequent PTEN and ras mutations, be estrogen receptor and progesterone receptor positive, display microsatellite instability, and express E-cadherin, whereas type II tumors generally display p53 mutations, are estrogen receptor/progesterone receptor negative, do not show microsatellite instability, and less commonly express E-cadherin (3, 4). Clear cell endometrial cancers display the characteristics of the type II pathway except for lower p53 expression than serous tumors and evidence of arising from atypical hyperplasia as well as endometrial intraepithelial carcinoma (5). It is unclear, then, whether the two-pathway paradigm accommodates clear cell endometrial cancer pathogenesis.

The pathogenesis of epithelial ovarian cancer is more poorly understood than that of endometrial cancer. Whereas the ovarian surface epithelium (OSE) is thought to be the source of most cases of ovarian cancer, evidence suggests that at least some endometrioid and clear cell ovarian cancers arise from

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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endometriosis (reviewed in ref. 6). A stepwise progression for ovarian carcinogenesis such as has been established for cervical and colorectal cancer has not been formulated, largely because of difficulty consistently documenting precursor lesions.

The uncertainty over the pathogenesis of ovarian cancer makes the possibility that the morphologic patterns it shares with endometrial cancer reflect common events in tumorigenesis intriguing. In fact, poor response rates for serous endometrial cancer to "standard" therapies for endometrial cancer such as doxorubicin combined with the clinical observation that serous endometrial cancer looks and acts like serous ovarian cancer have led to trials including first platinum and now taxanes, the mainstays of serous ovarian cancer chemotherapy, for the treatment of serous endometrial cancer (7).

In general though, treatment for ovarian and endometrial cancer is determined by the organ of origin with no regimens specific to histologic subtype. Clinicians have noted that the dismal response rate of clear cell ovarian cancer to chemotherapy (11-15%) contrasts sharply with that of serous ovarian cancer (72-73%), contributing to a 5-year survival for stage III to IV clear cell ovarian cancer of 20% compared with 32% for serous ovarian cancer (8, 9). The addition of platinum to the regimen did not lead to improved survival for clear cell ovarian cancer as it did for ovarian cancer as a whole (10, 11). Clear cell endometrial cancer likewise has a poor prognosis; whereas the overall 5-year survival for the disease is 75%, it is only 42% for the clear cell subtype (12). Currently, however, no alternative therapies have been discovered that target clear cell cancer more effectively, at least in part because trials are not structured to allow stratification of the results by histologic subtype. Thus, less data is available for rare subtypes such as clear cell and mucinous cancer.

In an effort to further define the molecular signatures of ovarian and endometrial cancer, we determined the gene expression profiles of serous, endometrioid, and clear cell cancers from both organs. In addition, we included renal clear cell cancer specimens for further comparison.

## Materials and Methods

**Tissue specimens.** A total of seven OSE brushings were obtained with a cytobrush from the normal ovaries of donors during surgery for other gynecologic disease at Tripler Army Medical Center according to an Institutional Review Board–approved protocol. Postmenopausal donors were selected, as this is the group in whom ovarian cancer most frequently arises. Of the four normal endometrial specimens, pathologic examination revealed atrophic endometrium in two patients, proliferative endometrium in one patient, and secretory endometrium in one patient. For quality assurance of the sample, the brush was first touched to a glass slide then immediately placed in Trizol (Invitrogen, Carlsbad, CA). The slide was later stained following a modified Papanicolaou protocol to confirm the epithelial content. Frozen samples of stage III or IV ovarian and endometrial carcinomas were obtained from the Cooperative Human Tissue Network and from patients at Memorial Sloan-Kettering Cancer Center according to an Institutional Review Board–approved protocol at the time of their initial staging operation. A total of 24 papillary serous, 11 endometrioid, and nine clear cell ovarian tumors were analyzed. A series of endometrial tumors including 19 papillary serous, seven endometrioid, and five clear cell cancers were also assessed. In addition, five renal clear cell carcinomas were used to resolve the relationships among clear cell cancers. All of the specimens were classified as high-grade (grade 3) and late-stage (stage III or IV) according to the International Federation of Gynecology and Obstetrics standards.

**RNA amplification and array preparation.** Total RNA was extracted from the normal brushings and the cancer samples using Trizol according to the manufacturer's instructions. An RNeasy kit was used to extract total RNA from the Memorial Sloan-Kettering Cancer Center cancer samples (Qiagen, Valencia, CA). RNA quality was confirmed by agarose gel electrophoresis. Universal Human Reference, which includes 10 human cell lines, was used as the reference (Stratagene, La Jolla, CA). Three micrograms of total RNA from each sample (including normals) were subjected to a single round of amplification using a modified Eberwine protocol.<sup>3</sup> The cDNA probes were prepared according to National Cancer Institute protocol.<sup>4</sup> Five micrograms of Cy3-labeled reference cDNA and Cy5-labeled sample cDNA were hybridized to an 11,000-element array produced by the National Cancer Institute Microarray Facility using Incyte UniGEM clones and ovary-specific genes from the Cancer Genome Anatomy Project. The array contains 9,896 genes, 1,306 of which represent expressed sequence tags. After 16 hours of hybridization, the arrays were washed and then scanned on an Axon 4000A laser scanner (Axon Instruments, Union City, CA).

**Microarray data analysis.** The scanned images were analyzed to determine raw expression levels after local background subtraction using GenePix Pro software (Axon Instruments). The BRB ArrayTools software<sup>5</sup> developed by Dr. Rich Simon and Amy Peng was used for analysis. After filtering out spots below the minimum signal intensity level of 250 counts or the minimum spot size of 50  $\mu$ m, 8,490 spots remained for analysis. A logarithmic (base 2) transformation was applied to the signal intensities, which were then normalized by median centering each array. Group classification was done by ANOVA using a stringent  $P = 0.001$  to limit the number of false positive results. Multiple permutations generated a  $P$  value to aid in assessing the reliability of the classification. False discovery rates were calculated using the false discovery controlling procedure of Benjamin and Hochberg (13). All of the reported gene lists possess a false discovery rate <20% except as noted. Class prediction was completed using leave-one-out cross-validation followed by permutation analysis for compound covariate, diagonal linear discriminant analysis, nearest neighbor classification, and the support vector machine with linear kernel predictor models. Principal component analysis was done after imputing missing values using Partek Pro 2000 software (Partek, Inc., St. Louis, MO). These data were imported into Matlab software (The Mathworks, Inc., Natick, MA) to allow depiction of the elliptic region where an additional sample of a particular group would fall with a 95% confidence level.

**Immunohistochemical analysis.** To determine p53 status, 5- $\mu$ m sections from tissue blocks of papillary serous, endometrioid, and clear cell ovarian and endometrial tumors included in the microarray analysis. These sections were processed as described previously (14). In addition, 5- $\mu$ m sections from an ovarian cancer tissue microarray were obtained from the GOG tissue bank to confirm the localization and protein expression level of caveolin-1 (CAV-1). Dewaxed tissue sections were immunostained using CAV-1 (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:50. Scorings of immunostained sections were based on the percentage of immunopositive cells (0-100) and staining intensity (0-4).

**Taqman PCR analysis.** Relative quantification of the six genes in each patient sample was determined using the comparative  $C_T$  method as follows. Specific primer pairs (assay on demand) for each gene were purchased from Applied Biosystems (Foster City, CA). The real-time PCR assays were done in 20- $\mu$ L reactions run on an ABI 7900 for 40 cycles (95°C for 15 minutes, 95°C for 15 seconds, 60°C for 30 seconds followed by a dissociation curve: 95°C for 15 seconds, 60°C for

<sup>3</sup> Amplification protocol available at <http://nciarray.nci.nih.gov/reference/index.shtml>.

<sup>4</sup> Probe preparation protocol available at <http://nciarray.nci.nih.gov/reference/index.shtml>.

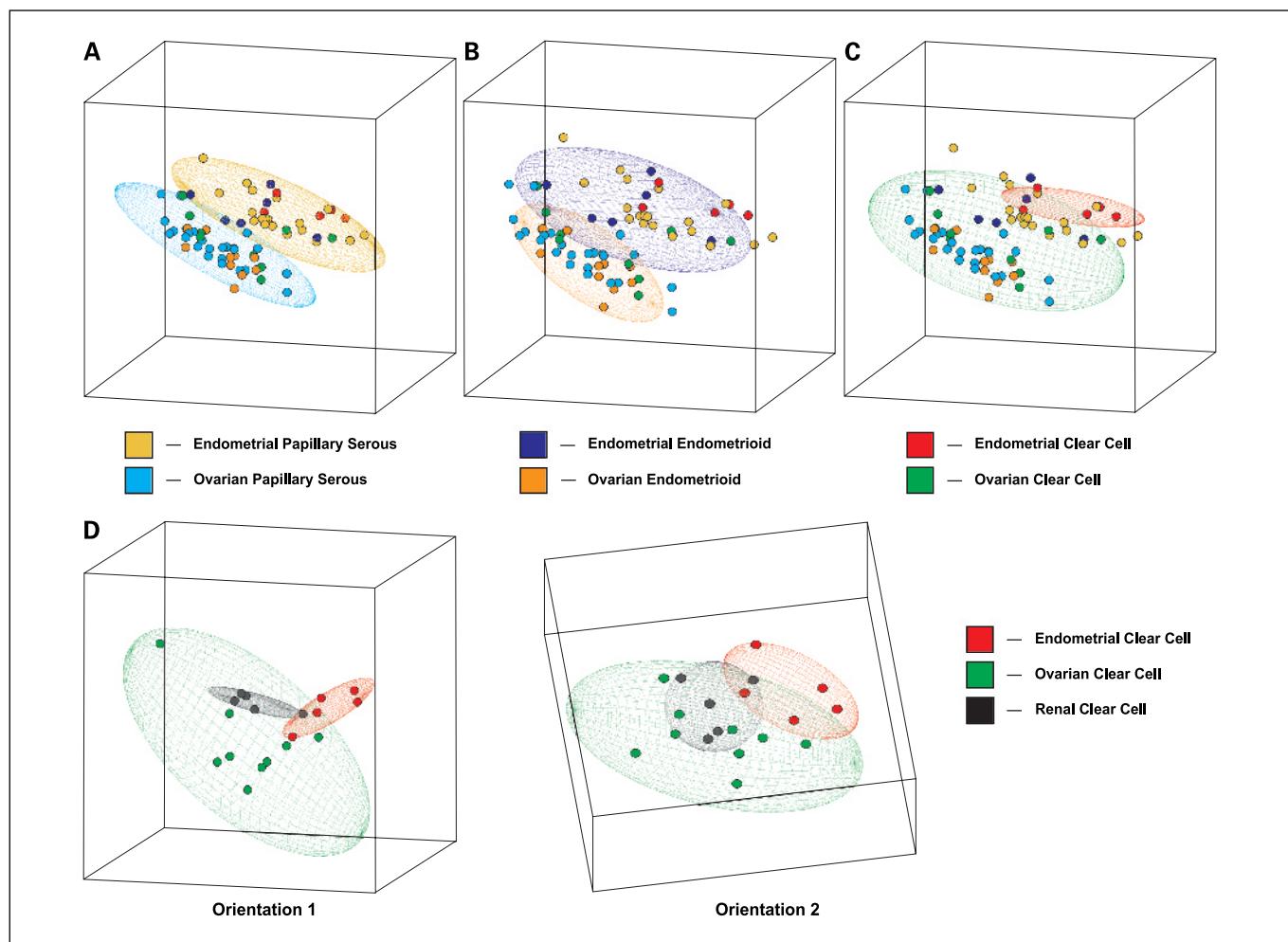
<sup>5</sup> BRB ArrayTools software available at <http://linus.nci.nih.gov/BRB-ArrayTools.html>.

15 seconds, 2% ramp rate to 95°C for 15 seconds). The relative expressions for the 14 genes were then normalized against the expression of CD74 such that the  $\Delta C_T$  values are equivalent to the average  $C_T$  value for each gene minus the average  $C_T$  value for CD74. CD74 was selected for normalization based on its consistent expression across all of the arrays selected for Taqman analysis (average ratio = 4.00, SD = 1.02). The results were comparable with the relative gene expression levels determined using the standard curve method.

## Results

**Comparison of ovarian, endometrial, and renal clear cell cancer.** We determined the gene expression profiles of 75 tumors representing analogous histologic subtypes (serous, endometrioid, and clear cell) of ovarian and endometrial cancers. To ensure a direct comparison, all specimens were advanced stage and poorly differentiated tumors. RNA was isolated from each tumor, amplified, profiled on cDNA microarrays. A direct comparison of their total gene expression patterns was made to evaluate similarities and differences among tumors.

Principal component analysis is a technique that allows the similarity of the gene expression profiles to be assessed by their proximity in three-dimensional space. To facilitate visualizing the relationships between the groups, an elliptic region was determined for each group such that an additional sample of that type would fall within the ellipse with a 95% confidence level. Figure 1 displays the relationships between the serous (A), endometrioid (B), and clear cell (C) subtypes of ovarian and endometrial cancer. The elliptic regions for serous and endometrioid samples are distinct; the clear cell samples, whereas clustering with their organ of origin, overlap more than the other subtypes. This suggested that the clear cell tumors could not be distinguished according to their organ of origin. To expand this observation, we profiled five renal clear cell tumors using the same platform. In Fig. 1D, the relationship among the clear cell samples in ovary, endometrium, and kidney are compared at two points in a three-dimensional space. Rather than forming an ellipse distinct from the gynecologic tumors as might be expected, the renal samples instead intermingle with them.



**Fig. 1.** Graphic depiction of principle component analysis of ovarian and endometrial cancers according to histology. Principle component analysis was done after imputing values using Partek Pro 2000 software (Partek). These data were imported into Matlab software (The Mathworks) to allow depiction of the elliptical region where an additional sample of a particular group would fall with a 95% confidence interval. *A*, analysis of tumors with serous histology showing two nonoverlapping elliptical regions separating endometrial (*top*) from ovarian (*bottom*) specimens. *B*, analysis of tumors with endometrioid histology showing two nonoverlapping elliptical regions separating endometrial (*top*) from ovarian (*bottom*) specimens. *C*, analysis of tumors with clear cell histology showing overlapping elliptical regions representing endometrial (*top*) and ovarian (*bottom*) specimens. *D*, analysis of tumors according to organ of origin shows three overlapping elliptical regions among ovarian, endometrial, and renal clear cell specimens. Two different orientations (1 and 2).

The dendograms produced by unsupervised hierarchical clustering of the samples also illustrate the relationships between the histologic subtypes. In Fig. 2A, the ovarian tumors clustered with the clear cell samples in a distinct branch interspersed with a few serous samples. The endometrioid and serous samples initially group together but then separate into one branch largely composed of endometrioid samples and another dominated by serous samples. Figure 2B depicts the endometrial tumors, with the clear cell samples again clustering tightly. The serous tumors divided even more clearly into a group that clusters with the clear cell samples and another that clusters with the endometrioid samples.

When the clustering was done based on histologic subtype, the serous (Fig. 3A) and endometrioid (Fig. 3B) samples form distinct ovarian and endometrial groups. The clear cell samples (Fig. 3C), however, did not group according to their organ of origin.

To substantiate the relationships between histology and organ of origin for ovarian and endometrial cancers, we applied a series of six class prediction tests to the 8,490 features used to generate the unsupervised dendrogram analysis (Table 1). The prediction error of each model was calculated using leave-one-out cross-validation by repeating the entire model building process for each training set. The performance of the classifier was returned as the percentage of arrays correctly classified. In addition, the cross-validated error rate was evaluated to determine whether the value was significantly less than one would expect from random prediction. This was completed by randomly permuting the class labels of the specimens and

repeating the leave-one-out cross-validation process 2,000 times. For the papillary serous and endometrioid histotypes, all six predictors successfully distinguished between the organs of origin ( $P < 0.001$ ). In contrast, for clear cell tumors only two of the six tests provided a significant predictor ( $P < 0.05$ ) indicating that the tumors could not be reliably assigned to a specific organ. Including a third set of clear cell renal tumors as an outlier class yielded all nonsignificant classifiers unable to distinguish the histologic subtype according to the organ of origin.

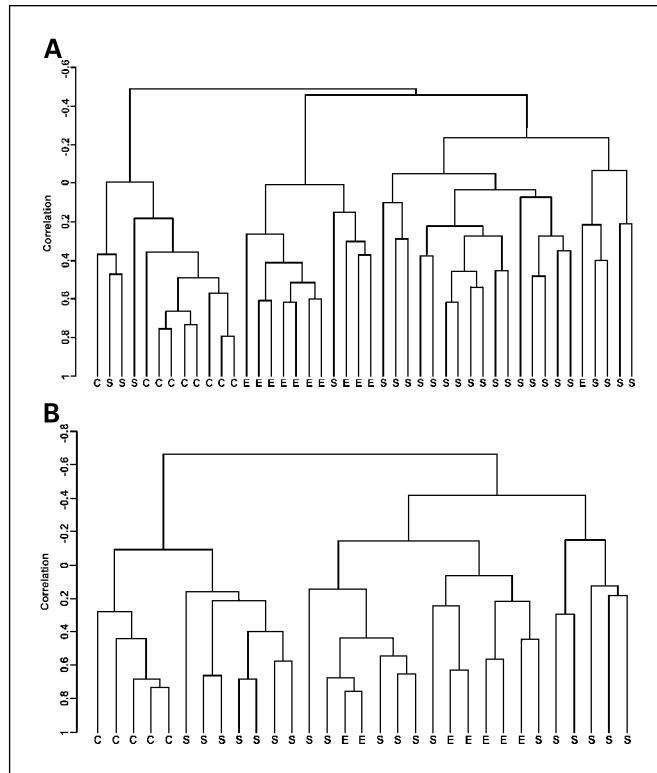
**Direct comparison of histologic subtypes of ovarian and endometrial cancer.** To determine the specific genes whose expression differentiates among these tumors, we compared each group directly. Comparing the subtypes of ovarian cancer directly to each at  $\alpha = 0.001$ , 166 genes differentiated the samples into the three subtypes. When clear cell ovarian cancer was compared with non-clear cell ovarian cancer (serous and endometrioid ovarian cancer grouped together), 171 genes were identified. Serous and endometrioid cancer were distinguished from the other histologic subtypes by 62 and 66 genes, respectively (see Supplementary Information).

Comparisons were also made between the profiles for endometrioid, serous, and clear cell endometrial cancer. At  $\alpha = 0.001$ , 70 genes discriminated between the three subtypes. Fifty genes distinguished clear cell histology from serous and endometrioid endometrial cancer considered as a group. A comparison of endometrioid cancer to the other subtypes generated a list of 58 genes (see Supplementary Information).

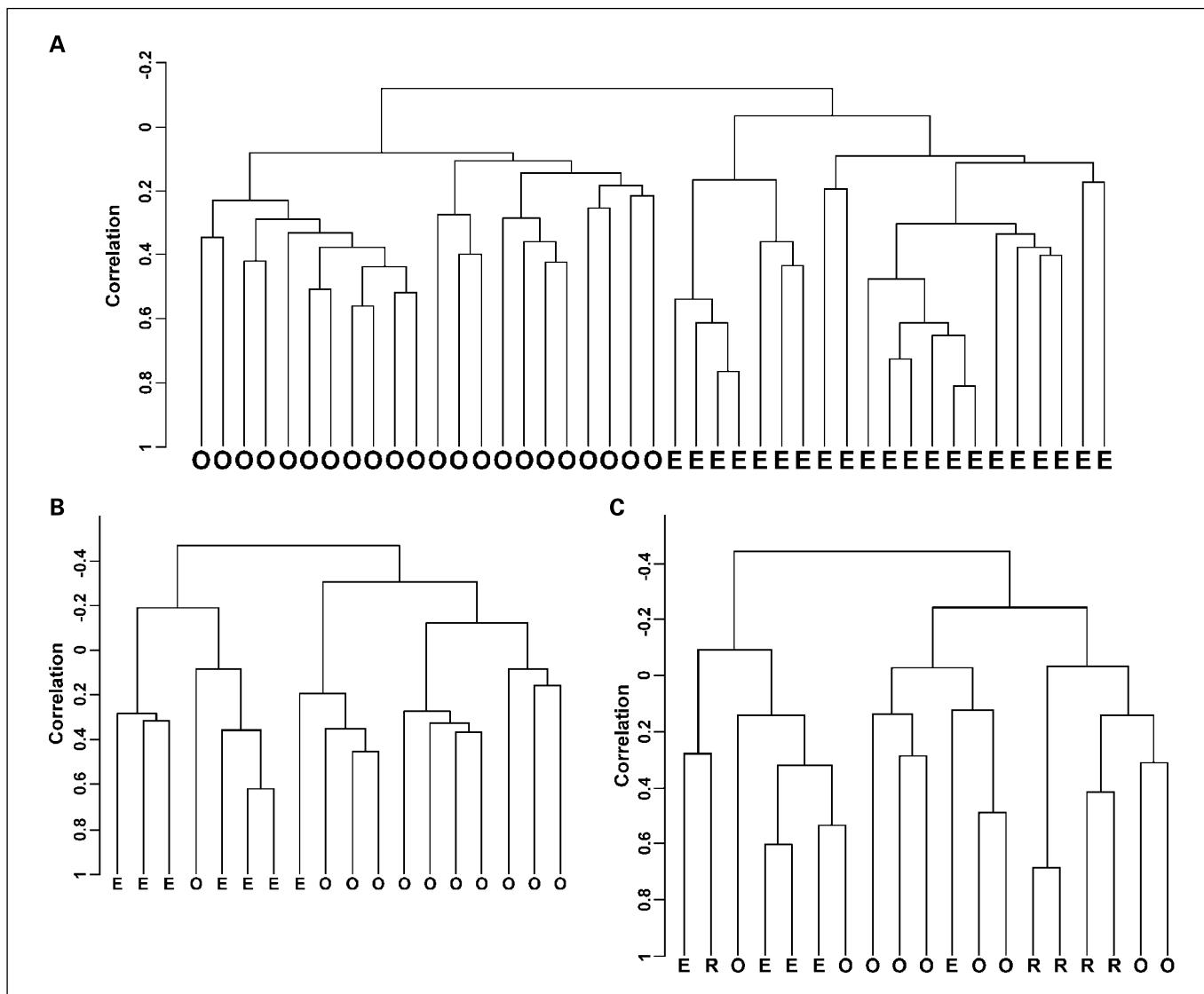
Subsequently, the lists of genes that defined each subtype in ovarian and endometrial cancer were compared. Of note, only the clear cell analysis produced a group of genes differentially expressed in both ovary and endometrium (Table 2). Included in this clear cell profile are FXYD domain-containing ion transport regulator 2 (*FXYD2*), tissue factor pathway inhibitor 2 (*TFPI2*), Annexin A4 (*ANXA4*), glutaredoxin (*GLRX*), mitogen-activated protein kinase kinase kinase 5/apoptosis signal-regulating kinase 1 (*MAP3K5/ASK1*), and UDP glycosyltransferase 1 family, polypeptide A1 (*UGT1A1*).

The 166-gene ovarian cancer list and the 70-gene endometrial cancer list that separated the samples into three subtypes were also compared. Nine genes appeared on both the endometrial and ovarian cancer lists (Table 3). Interestingly, five of the nine also are found on the clear cell profile (*FXYD2*, *GLRX*, *ANXA4*, *UGT1A1*, and *MAP3K5*).

**Comparison of ovarian and endometrial cancers with their normal tissue counterparts.** To identify genes involved in the development of ovarian and endometrial tumors, separate comparisons of each histologic subtype to normal OSE brushings or normal endometrium brushings were completed. For each comparison, tumor and normal expression values were averaged independently. Ovarian cancers evaluated with  $\alpha = 0.001$  yielded lists of 94 genes for clear cell cancer, 422 genes for endometrioid cancer, and 467 genes for serous cancer (see Supplementary Information). Forty-three genes were common to all three lists; these genes, therefore, displayed consistent differential expression between normal OSE and ovarian cancer regardless of histologic subtype (see Supplementary Information). The majority of the genes are known, whereas 15 represent expressed sequence tags, including five genes from the CGAP ovary library. Twenty-nine genes have increased expression in ovarian cancer compared with normal OSE, whereas 14 have decreased expression in cancer. Among



**Fig. 2.** Dendograms produced by unsupervised hierarchical clustering of the (A) ovarian samples and (B) endometrial samples. Abbreviations: S, serous histology; E, endometrioid histology; C, clear cell histology.



**Fig. 3.** Dendrograms produced by unsupervised hierarchical clustering according to (A) serous histology, (B) endometrioid histology, and (C) clear cell histology. Abbreviations: O, ovarian samples; E, endometrial samples; R, renal samples.

the genes with increased expression in cancer are homogentisate oxidase (*HGD*), peroxisome proliferative activated receptor gamma (*PPARG*), v-rel reticuloendotheliosis viral oncogene homologue B (*RELB*), and p21-activated kinase 1 (*PAK1*). Decreased expression was documented for tenascin XB (*TNXB*), galectin 8 (*LGALS8*), post-meiotic segregation increased 2-like 8 and 2-like 9 (*PMS2L8* and *PMS2L9*), deafness autosomal dominant 5/inversely correlated with estrogen receptor expression 1 (*DFNA5/ICERE1*), disabled homologue 2/differentially expressed in ovarian cancer 2 (*DAB2/DOC2*), and retinoic acid receptor responder 1 (*RARRES1/TIG1*). Whereas *HGD* and *DAB2/DOC2* have been found previously associated with ovarian cancer, the others have not (15–17). When the subtypes were grouped into ovarian cancer as a whole, the comparison with normal OSE yielded 455 genes, many of which overlapped the subtype-normal comparisons as expected (see Supplementary Information). Examples of genes that did not appear on all the subtype lists are Kirsten rat sarcoma 2 viral oncogene homologue (*KRAS2*), claudin 1/

senescence-associated membrane protein 1 (*CLDN1/SEMP1*), and neurexin 1 (*NRXN1*).

When each endometrial cancer histologic subtype was separately compared with the normal endometrial brushings at  $\alpha = 0.001$ , only 24 genes differentiated endometrioid cancer from normal endometrium (false discovery rate = 37.5%). The corresponding gene lists for serous and clear cell cancer were 253 and 446, respectively, suggesting much more distinct profiles (see Supplementary Information). Four genes were present on all three lists, including *CLPP*, *RASA1*, *CIRBP*, and *ID1*; however, 40 genes appeared on the comparisons of both serous and clear cell cancer with normal endometrium. When endometrial cancers (as a whole) were compared with normal endometrium, the differentially expressed gene list identified 173 genes (see Supplementary Information).

When the lists of genes that distinguished ovarian and endometrial cancer as a whole from their respective normal tissues were compared, only 13 genes were found on both (see Supplementary Information). Among these genes are *CAV-1*

**Table 1.** Class prediction analysis comparing histologic subtypes for ovarian and endometrial cancer including clear cell renal tumors

|                        | Compound covariate predictor ( <i>P</i> ) | Diagonal linear discriminant analysis ( <i>P</i> ) | 1-Nearest neighbor ( <i>P</i> ) | 3-Nearest neighbors ( <i>P</i> ) | Nearest centroid ( <i>P</i> ) | Support vector machines ( <i>P</i> ) |
|------------------------|---|--|---------------------------------|----------------------------------|-------------------------------|--------------------------------------|
| Papillary serous       | 93% (<5e <sup>-4</sup> )                  | 95% (<5e <sup>-4</sup> )                           | 97% (<5e <sup>-4</sup> )        | 93% (<5e <sup>-4</sup> )         | 93% (<5e <sup>-4</sup> )      | 100% (<5e <sup>-4</sup> )            |
| Endometrioid           | 94% (<5e <sup>-4</sup> )                  | 94% (<5e <sup>-4</sup> )                           | 83% (0.006)                     | 83% (0.005)                      | 94% (0.001)                   | 94% (<5e <sup>-4</sup> )             |
| Clear cell             | 86% (0.024)                               | 79% (0.073)  | 71% (0.206)                     | 71% (0.348)                      | 86% (0.017)                   | 79% (0.069)                          |
| Clear cell with renal* | —   | —  | 53% (0.701)                     | 53% (0.659)                      | 47% (1.0)                     | —                                    |

NOTE: Percentage of arrays correctly classified after leave-one-out cross-validation is indicated along with the cross-validated error rate *P* value.

\*Only 1-nearest neighbor, 3-nearest neighbor, and nearest centroid predictors support the classification of more than two distinct classes.

and *DFNA5/ICERE1*, which displayed decreased expression in ovarian and endometrial cancer. HGD also appeared on the list but its expression pattern differed between the two organs; HGD expression was up in ovarian cancer compared with normal but down in endometrial cancer compared with normal. The remainder of the genes on each list was unique to the ovarian or endometrial transformation process.

Because it is well known that a high percentage of high-grade serous and endometrioid tumors have p53 mutations, it is possible that differentially expressed genes in these tumors might be p53 regulated. We determined the p53 status of these tumors by immunohistochemical staining: (a) For serous specimens, a total of 13 ovarian samples of 24 stained positively for mutant p53, whereas 12 of 19 endometrial tumors were positive. (b) Among the endometrioid samples, 3 of 11 ovarian tumors were positive, and all seven endometrial cancers were negative.

Microarrays corresponding to the positive and negative staining specimens were partitioned for each histologic subtype according to the organ of origin and reanalyzed comparing each group with the appropriate normal. Differentially regulated genes were then cross-compared with the gene lists that distinguished the three histotypes. For all eight subtype-specific gene lists, none of the genes associated with p53 status were identified.

**Taqman validation of microarray data.** Quantitative real-time PCR was used to validate the microarray data for genes with increased expression in ovarian cancer relative to normal OSE (*PPARG*, *RELB*, *PAK1*, and *MAPK8/JNK1*) as well as genes with higher expression in clear cell ovarian and endometrial

cancer compared with the other subtypes (*TFPI2* and *FXYD2*). The assays were run in a 41-sample subset of the array project. The relative gene expression ratios seen in the array data for *PPARG*, *RELB*, *PAK1*, and *MAPK8/JNK1* were confirmed by the PCR assays. Similar, the overexpression of *TFPI2* and *FXYD2* in the clear cell subtype on the arrays was confirmed by the PCR data, although the fold-difference was generally higher in the PCR assays (see Supplementary Information).

**Localization and validation of caveolin-1 protein expression.** To determine the ovarian cell subpopulation(s) expressing CAV-1, five paraffin-embedded sections of a tissue microarray containing normal ovarian tissue and ovarian cancers of different histologies were analyzed using immunohistochemical staining. Normal tissue was chosen to establish cellular localization, because CAV-1 was down-regulated in all three ovarian histotypes. Marked expression of the gene was observed in epithelial cells with 50% to 90% cells staining at an intensity of 3 or 4 (10-50% of stromal cells also stained at an intensity of 3 or 4) for all five specimens (see Supplementary Data). In contrast, two serous tumors displayed weak, diffuse tumor cell staining (<30%), whereas the remaining samples lacked any detectable levels of protein. Moderate stromal staining was observed for three (<50%) endometrioid tumors and a single (<50%) clear cell specimen at intensity values of 3 or 4.

## Discussion

Analogous histologic subtypes of tumor found in endometrial and ovarian cancers have raised theories concerning potential similar developmental origins and/or differentiation

**Table 2.** Genes common to ovarian and endometrial clear cell tumors

| Description   | Locus link ID | Gene symbol     | Map     |
|---|---------------|-----------------|---------|
| Tissue factor pathway inhibitor 2                           | 7980          | <i>TFPI2</i>    | 7q22    |
| Annexin A4  | 307           | <i>ANXA4</i>    | 2p13    |
| UDP glycosyltransferase 1 family, polypeptide A1            | 54658         | <i>UGT1A1</i>   | 2q37    |
| FXYD domain containing ion transport regulator 2            | 486           | <i>FXYD2</i>    | 11q23   |
| Glutaredoxin (thioltransferase)                             | 2745          | <i>GLRX</i>     | 5q14    |
| KIAA1922 protein  | 114819        | <i>KIAA1922</i> | 1p36.13 |
| Mitogen-activated protein kinase kinase kinase 5            | 4217          | <i>MAP3K5</i>   | 6q22.33 |
| Coxsackie virus and adenovirus receptor                     | 1525          | <i>CXADR</i>    | 21q21.1 |
| Regulatory factor X, 5 (influences HLA class II expression) | 5993          | <i>RFX5</i>     | 1q21    |

**Table 3.** Genes common to ovarian and endometrial cancers that are able to distinguish among histotypes

| Description   | Locus link ID | Gene symbol   | Map            |
|---|---------------|---------------|----------------|
| Microphthalmia-associated transcription factor        | 4286          | <i>MITF</i>   | 3p14.1-p12.3   |
| Annexin A4  | 307           | <i>ANXA4</i>  | 2p13           |
| UDP glycosyltransferase 1 family, polypeptide A1      | 54658         | <i>UGT1A1</i> | 2q37           |
| FXYD domain containing ion transport regulator 2      | 486           | <i>FXYD2</i>  | 11q23          |
| Glutaredoxin (thioltransferase)                       | 2745          | <i>GLRX</i>   | 5q14           |
| Degenerative spermatocyte homologue, lipid desaturase | 8560          | <i>DEGS</i>   | 1q42.12        |
| Mitogen-activated protein kinase kinase kinase 5      | 4217          | <i>MAP3K5</i> | 6q22.33        |
| NGFI-A binding protein 1 (EGR1 binding protein 1)     | 4664          | <i>NAB1</i>   | 2q32.3-q33     |
| Basic helix-loop-helix domain containing, class B, 3  | 79365         | <i>BHLHB3</i> | 12p11.23-p12.1 |

programs of these tumors. In addition, these similar histologies have raised potentially important questions concerning the appropriate clinical management of these tumors. We chose to explore these issues by comparing the molecular basis of these tumors using expression profiling. Gene expression profiles can serve as powerful tools to determine biological relationships between tumors. The comparison of the gene expression profiles of the endometrioid and serous subtypes of ovarian and endometrial cancer produced gene lists that are largely unique to the combination of a particular subtype in a specific organ. In contrast, clear cell cancers show a remarkable similarity in gene expression profiles across organs (including kidney) and could not be statistically distinguished. In addition, the differentially expressed genes among these tumor types and their normal counterparts provide insights into the tumors' origins and clinical behavior.

A group of 43 genes appeared on each ovarian cancer subtype's comparison with normal OSE. This suggests that at least part of the transformation process is shared among endometrioid, serous, and clear cell ovarian cancer, as evidenced by the common genes distinguishing them from normal OSE. However, the question of whether the OSE serves as a common precursor is not clarified. It is conceivable that tumors of different histologies may arise from different precursor cells but undergo similar transformation events. For endometrial cancers, only four genes appeared on all three lists for endometrial cancer. This suggests a more diverse origin for endometrial cancers. This observation could be interpreted as supporting the theory that two pathways to endometrial malignancy exist, one for endometrioid endometrial cancer and another for the serous and clear cell subtypes. However, serous and endometrioid endometrial cancer did not segregate in either hierarchical clustering or principal component analysis, as the theory of carcinogenesis would suggest and as has been shown in a previous array study (18). The relatively indistinct profile obtained for the endometrioid endometrial cancer group is likely the reason. Our late-stage, high-grade endometrioid samples likely represent a relatively dedifferentiated group compared with the signature tumor for endometrioid endometrial cancer which is localized, superficially invasive, and well differentiated. The fact that the previous study did not specifically select for grade and stage suggests that their endometrioid samples were low-stage and low-grade, whereas their serous and clear cell samples were late-stage and high-grade, because these are the most common presentations

of those subtypes. Our endometrioid cancers of the endometrium likely represent a heterogeneous set of tumors, some of which have gene expression similarities to serous and clear cell cancers and a subset of which reflect different developmental origins. This may also explain differences in the gene lists derived in this study when compared with those reported in other studies focusing on well-differentiated endometrial cancers (19–21). In both studies, the differentially expressed genes that were identified were all hormonally regulated, as one would expect in well-differentiated tumors.

Direct comparison of tumors with different histologies using a single genomic platform allows for comparisons and observations, which may have clinical implications. Although it has been postulated that papillary serous tumors of the ovary and endometrium are essentially identical tumors, our analysis could not identify a gene set that distinguish serous cancers (across organ site) from endometrioid and clear cell subtypes. This suggests that these tumors have important gene expression differences, which makes it less likely that they can be clinically managed in an identical fashion. It is possible that a subset of serous endometrial cancer may exist that more closely resembles serous ovarian cancer, a group that might respond well to an "ovarian" regimen based on platinum and taxanes. Studies involving larger numbers of specimens will be needed to confirm that.

The mechanism(s) of differential expression of these genes remains unknown. Analysis of the gene expression patterns according to p53 status showed that the expression of these genes did not correlate with the mutational status of this tumor suppressor gene. There are two possible explanations for this: (a) The complex transcriptional regulation of these genes makes it unlikely to be effected by one transcription factor. (b) Although only a portion of our high-grade tumors had mutations by immunohistochemistry, it is highly likely that the p53 pathway is affected in a much higher percentage of these tumors.

Conversely, our data on clear cell carcinomas shows a remarkable similarity, which showed differential expression of a small set of genes in both ovary and endometrium. The relatively high correlation in our study of ovarian and endometrial clear cell cancer with renal clear cell cancer samples suggests that certain molecular events may be common to clear cell tumors regardless of the organ of origin. These results extend the findings of Schwartz et al. and Schaner et al., which showed a distinct molecular signature for clear cell

ovarian cancer (16, 22). These common features may reflect a shared precursor cell or common transformation pathways. The former may be more likely than the latter, because we know there are some activated pathways present in renal clear cell cancers (such as VHL), which are not present in clear cell cancers from the ovary or endometrium.

The comparison of expression profiles of cancer with their normal counterparts identified multiple genes, which may play a role in the development of these cancers. Examination of the gene lists revealed the presence of several genes whose expression patterns implicate them as potential tumor suppressors. CAV-1 displayed lower expression in both ovarian and endometrial cancer relative to their respective normal counterparts. CAV-1 expression has been found to be down-regulated in breast and lung cancer cell lines in the past, with recent confirmation of its down-regulation in lung, thyroid, and ovarian cancer as well (23–25). The development of a knockout model for CAV-1 has provided evidence for a tumor suppressor role in mice (26, 27). We showed decreased expression of the protein in ovarian tumors of all three histologies relative to normal ovarian tissue confirming the microarray data. Furthermore, localization of the protein to ovarian epithelium suggests this protein may play a role in ovarian tumor development. Two other potential tumor suppressor genes, *DAB2/DOC2* and *RARRES1/TIG1*, decreased expression in all ovarian cancer subtypes relative to normal OSE. Previous research documented decreased expression of *RARRES1/TIG1*, thought to be a cell adhesion molecule, in malignant prostate samples relative to both normal prostate tissue and benign prostatic hyper trophy samples (28, 29). The decreased expression of *DOC2* in cancer confirms previous reports in ovarian, colorectal, pancreatic, prostate, pulmonary, and mammary carcinomas, as well as choriocarcinoma (17, 23, 30). However, *DOC2* expression was also down in serous endometrial cancer compared with normal endometrium, which has not been described previously. It is important to note that the validation of these differentially expressed genes will require the determination of protein levels by

Western blot or immunohistochemical staining to substantiate their biological role in the tumor.

Among the genes included in the profiles of both ovarian and endometrial clear cell tumors were *FXYD2*, *TFPI2*, *ANXA4*, and *GLRX*, all of which previously have been reported to be associated with clear cell ovarian cancer (16). *MAP3K5/ASK1* also appears in the clear cell profile; along with *GLRX*, it is involved in regulation of apoptosis via *JNK1* (31). Two of the genes have intriguing associations with chemotherapy response: *ANXA4* has been associated with paclitaxel resistance, whereas *UGT1A1* detoxifies the active metabolite of irinotecan, SN-38 (32, 33). The finding that *FXYD2*, *GLRX*, *ANXA4*, *UGT1A1*, and *MAP3K5* also dominated the list of genes that distinguish the three subtypes in both organs reinforces that a set of genes exists that defines the clear cell subtype, whereas comparable common genes cannot be found for the serous and endometrioid subtypes in this analysis. The sum total clinical/biological effect of these genes is to increase apoptotic signals (*ASK1/GLRX*), inhibit cellular proliferation (*TFPI2*), and increase resistance to chemotherapeutic agents (*ANXA4* and *UGT1A1*), which is consistent with the slow growth and chemoresistance of clear cell tumors.

If interventions can be designed based on these differentially expressed genes or activated pathways specific for clear cell cancers, treatment for at least this small subset of ovarian and endometrial cancer may be directed by histologic subtype in the future, replacing the current organ-based approach. The failure of current chemotherapy regimens for clear cell cancer is recognized. Many oncologists consider experimental therapies first line for renal clear cell cancer in light of poor success with standard cytotoxic regimens. Clear cell ovarian and endometrial cancer is associated with decreased survival relative to ovarian and endometrial cancer as a whole (8, 9, 12). To identify better therapies for clear cell cancer, future trials may need to enroll primarily based on the presence of clear cell histology rather than the location of the tumor.

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